HEMOLYSIS AND BLOOD CONCENTRATION OF SULFONES "IN VIVO"

By G. ROSENFELD, H. RZEPPA, L. NAHAS and S. SCHENBERG
(Laboratory of Hematology, Instituto Butantan, S. Paulo, Brazil)

The anemia induced by diamino-diphenyl sulfone (DDS) has been observed by many authors who have employed this drug chiefly on the treatment of leprosy. Faget *et al.* 1943 (5) observed that "Promin" in daily oral doses of 0.5-1.0 gm frequently caused toxic reactions and a marked hemolysis that compelled to interrupt treatment. Intravenously the drug was less toxic and could be used in daily doses from 1 to 5 gm which provoked anemia in 46 % of the cases. Fernandez *et al.* (6) in 140 patients treated with "Diazone", noted that daily oral doses of over 1 gm caused a drop of the red blood cell count and hemoglobin in the second week of treatment. Lowe (8) found the same anemia using daily doses larger than 300 mg of DDS, with a blood concentration varying between 1 to 1.8 mg %. Smith (11) noticed anemia in patients treated by mouth with "Diazone" in daily doses of over 2.4 gm. With DDS the toxic manifestations appeared with daily doses of 300 mg (10). Allday and Barnes (1) found toxic effects using DDS orally in daily doses of 200 mg during 2 to 8 weeks but they did not consider the hypochromic anemia as an important symptom. In patients treated orally with 0.6 to 0.9 gm of "Diazone" ** and 0.2 to 0.4 gm of DDS daily, Souza Lima, Nahas and Rzeppa (9) described an anemia that appeared when the blood concentration of the drug was greater than 0.6 mg %. This concentration was obtained with doses of 0.4 gm per day of DDS and exceptionally with "Diazone" in doses of 0.9 gm.

Table 1 shows the data from different authors that agree in respect to the dosis of DDS which causes anemia and which is greater than 300 mg per day. However these opinions differ about the mechanism of the anemia and at present it is not possible to conclude anything about it. Faget (5) does not present any data about the probable nature of this anemia, although he refers to a hemolysis which may be corrected by the use of antianemics. In refractory cases to such therapy the anemia is corrected by the interruption of treatment.

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** Synthetized in Instituto Butantan under the name "Diaminoxil".
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TABLE 1

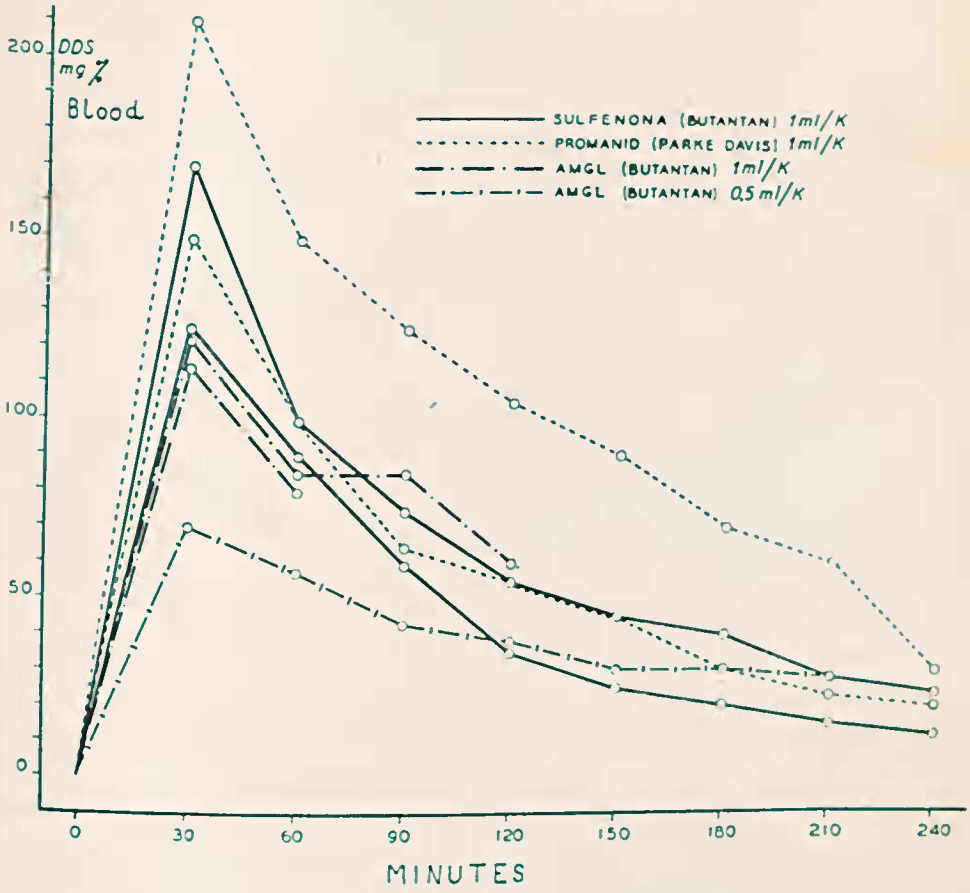
Relation between doses of sulfone, blood concentration and anemia (irom literature data) *Results were also calculated in DDS, in order to facilitate the comparison*

Author	Drug	Administration Way	Daily dosis gm.	D D S mg.	Blood concentration of DDS mg.%	Anemia
Faget & Col. (5)	Promin	Intravenous	1-5	310-1.550	—	+
Faget & Col. (5)	Promin	Orally	0.5-1.0	155- 310	—	+
Lowe (8)	D D S	Orally	> 0.3	> 300	1.0-1.8	—
Allday & Col. (5)	D D S	Orally	0.2	200	—	—
Souza Lima & Col. (9)	D D S	Orally	0.2	200	< 0.6	+
Souza Lima & Col. (9)	D D S	Orally	0.4	400	> 0.6	—
Souza Lima & Col. (9)	Diazone	Orally	0.6	330	< 0.6	+
Souza Lima & Col. (9)	Diazone	Orally	0.9	495	< 0.6	+
Fernández & Col. (9) ..	Diazone	Orally	> 1.0	555	—	+
Smith (11)	Diazone	Orally	> 2.4	> 1.320	—	+

Fernandez *et al.* (6) consider this anemia of the hemolytic type due to the following findings: 1) reticulocytosis; 2) increase of the biliary pigments in urine; 3) increase of bilirubin in blood; 4) slight increase of the fragility of red blood cells. Smith (2) contradicts these authors because of these findings, which may be found in other types of non-hemolytic anemias, chiefly when they are not very marked. He concludes that the anemia induced by sulphones has a complex mechanism where hemolysis which is detected by the bilirubinemia and slight increase of the fecal urobilin is the less important fact.

Brownlee (4) using rabbits whose diet contained 4 % of "sulphetrone" concludes that their anemia is produced by three mechanisms: 1) slight but continuous hemolytic anemia marked by the concurrent reticulocytosis; 2) lack of iron due to competition of the alimentary iron with sulphetrone resulting a non-absorbable complex; 3) anemia of nutritional origin caused probably by limitation and alteration of the bacterial flora of the gut by sulphetrone. This anemia may be prevented by the simultaneous administration of brewers yeasts. Higgins (7) does not reach clear conclusions about the nature of the anemia that he observed in guinea pigs treated with 200 mg of "Promin" by mouth. In spite of the resemblance between these blood changes and those of hemolytic anemia, he noticed increase of the fragility of red blood cells and no spherocytosis.

As the conclusions of the literature are discordant in respect to the mechanism of anemia pointed by several authors, our purpose is to verify whether the DDS has an hemolytic action *in vivo* when large doses of sulphones are injected to dogs.



GRAPH 1

DDS blood concentration in dogs injected with different sulfones

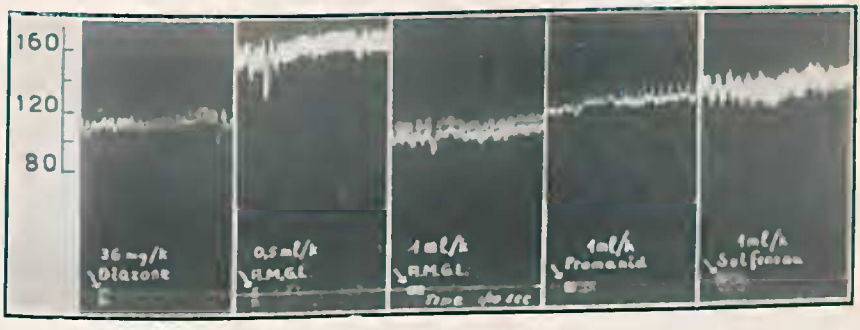


FIG. 1

Graphical record of carotidial pressure in dogs injected with different sulfones. Only the AMGL has a little transitory hypotensive action.

MATERIAL AND METHODS

In dogs anesthetized with nembutal (35 mg per kg of body weight by the intraperitoneal route) hematological examinations and determination of DDS in blood by Brownlee's method (3) as modified by Rzeppa (9) were made. The hematological examinations were made in venous heparizized blood (0.1 mg per ml of blood). Hemolysis was estimated by determination of plasma hemoglobin. For that purpose, the supernatant plasma of the hematocrit, diluted 1 to 20 (0.25 ml of plasma plus 4.75 ml of saline solution) was used. The hemoglobin concentration of plasma was calculated in relation to the concentration of total hemoglobin of the same sample of blood. The result showed the percentage of hemolysis. The determinations of hemoglobin were made in a spectrophotometer. The first determination was done in the anesthetized dog immediatly before the intravenous injection of drug. The following determinations were done at intervals of 30 minutes for 300 minutes after the injection. The drugs employed were:

"Diazone" (4,4' diamino-diphenyl-sulfone N,N' bis methylene sulphoxilate of sodium) in 40 % solution. Synthetized at the Chemotherapy Section of the Instituto Butantan (2) under the name of "Diaminoxil".

"Promin" (4,4' diamino-diphenyl-sulfone N,N' bis glucose sulfonate of sodium) in 40 % solution. Synthetized at the Chemotherapy Department of the Instituto Butantan (2) under the name of "Sulfenona".

"Promin" (4,4' diamino-diphenyl-sulfone N,N' glycose sulfonate of sodium) in 40 % solution prepared by Parke Davis under the name of "Promanid".

"AMGL" (4,4' diamino-diphenyl-sulfone N,N' bis glycose) in 30% solution. Synthetized at the Chemotherapy Department of the Instituto Butantan (2).

RESULTS

Doses five times larger than those used for human therapy have been employed. The use of these larger doses was made with the purpose disclosing if the drug has really a direct hemolytic action *in vivo*. This was clearly demonstrated as can be seen in Table 2.

The hemolysis was not proportional to the concentration of DDS injected and it depends upon the salt employed. Thus the DDS under the form of "Diazone" in concentration of 30 mg/kg induced a hemolysis comparable to that produced by 130 mg/kg of "AMGL" and 125 mg/kg of "Promin". The "AMGL" showed to be more hemolytic than "Promin" because in doses of 65 mg/kg it produced a hemolysis like that provoked by 125 mg/kg of "Promin".

TABLE 2

Dogs injected intravenously with several sulfones. Hemolysis represented by percentage of plasma hemoglobin in relation to total hemoglobin of blood.

Dog	Drug injected	mg./K	DDS ml/k	Hemolysis %									
				Time in minutes after the injection									
				Before	30	60	90	120	150	180	210	240	300
4-52-S	Diazone 1.805 k.%	2	20	0.0	0.48	0.48	0.48	1.97	—	1.33	1.33	3.7	—
8-52-S	AMGL 30 %	1	130	0.0	1.18	1.18	1.13	1.13	4.45	3.8	2.6	—	—
9-52-S	AMGL 30 %	1	130	0.0	0.84	0.84	—	—	—	3.3	2.8	—	—
10-52-S	AMGL 30 %	1	65	0.0	0.0	1.6	1.6	1.7	2.3	—	—	—	—
12-52-S	Promanid 40 %	0.5	125	0.0	1.11	2.0	2.0	2.0	2.6	—	—	300	1.52
13-52-S	Sulfenona 40 %	1	125	0.0	0.24	0.8	0.8	1.28	1.33	—	—	240	—

At 150 minutes, in the concentration of 130 mg/kg the "AMGL" provoked a hemolysis greater than the one produced by 125 mg/kg of "Promin".

The "Promin" made in Instituto Butantan, when compared with "Promin" of Parke Davis shows itself to be less hemolytic, which may be interpreted as a greater stability of the salt and consequently smaller release of DDS.

From these data it is clear that DDS is directly hemolytic *in vivo* and that its hemolytic power seems directly proportional to the velocity with which the salt employed can liberate the DDS as "Diazone" is more easily hidrolyzed than "AMGL" and the latter more than "Promin".

Since up to now there is no method for determining the amount of free DDS in the blood, hemolysis happens to be an acceptable indicator to this concentration. It can also show the path for researches about the employed salts therapeutic qualities as it seems that the more active the drugs, the more easily they liberate the DDS. The latter can be measured by the degree of hemolysis.

In graph 1 it can be seen that great amounts of drug only cause transitory rise of the concentration because 4 hours after venous injection the levels are much lower and resemble those obtained when smaller doses which do not reach such high levels are injected. Equal doses of DDS result in different maximum levels according to the salt injected. Thus with "Promin", a higher concentration than that with "AMGL" is obtained.

The hemolysis is not proportional to the acme of the drug concentration because both "Promins" caused less hemolysis than "AMGL" in spite of transitory higher concentration.

In the doses employed the drugs did not have action over the of red blood cells count, hematocrit, mean corpuscular volume, sedimentation rate und leucocytes count.

"Diazone", "Promanid" and "Sulfenona" when injected intravenously did not produce any change of carotidial pressure in the dog. "AMGL" induced a very slight transitorial fall of pressure, 1 minute after being injected (fig. 1).

In order to verify if "Promin" injections in large amounts did produce thrombosis, volumes of 8 to 16 ml, containing 3.2 to 6.4 gm, were injected in femoral vein of dogs. After injected they were totally bleeded and autopsiated. No thrombosis was found in the vessels neither in the heart.

SUMMARY

Several authors who have employed diamino-diphenyl-sulfone (DDS) observed that the use of this drug led to the development of anemia. Opinions differ as to the mechanism of anemia, some suggesting that it is hemolytic because of the increase of reticulocytes, increase and elimination of bilirubin.

Others are against this belief on account of the irregularity of such findings.

In order to verify whether such sulfones are directly hemolytic this drug has been injected in dogs intravenously in doses 5 times larger than those therapeutically applied so as to have a better way to judge its eventual hemolytic action.

Hemolysis was observed in all animals, the intensity varying in accordance with the drug employed. The hemolysis increased until the 4th hour, without diminution after this period. "Diazone" was observed to be more hemolytic than the "AMGL", and the latter more than the "Promin". On the other hand, it seems that the hemolysis is directly proportional to the concentration of the drug, giving for this an evaluation of DDS free in the blood for what does not exist yet any method of assay.

The drugs utilized in the observed periods of time had no influence on the number and mean corpuscular volume of the red blood cells or on the leucocytes. They also did not provoke alteration on the carotidial pressure with exception of the AMGL which had a little and transitorial depressive action.

No thrombosis was found when large amounts of "Promin" were injected in the femural vein of dogs.

RESUMO

A anemia provocada pela diamino difenil sulfona (DDS) tem sido assinalada por vários autores que usaram êsse medicamento principalmente na lepra. Quanto ao mecanismo dessa anemia há opiniões discordantes, sugerindo alguns que é hemolitica devido ao aumento de reticulocitos, aumento da eliminação de bilirrubina, no que são contestados por outros pela irregularidade desses achados.

Com o fim de verificar si essas sulfonas são diretamente hemoliticas injetou-se em cães por via venosa várias sulfonas em doses 5 vezes maiores do que as terapêuticas afim de evidenciar melhor a eventual ação hemolitica.

Observou-se em todos os animais uma hemólise que variou de intensidade com a droga e que já se iniciava 30 minutos após a injeção, aumentando até a 4.ª hora sem regredir no fim desse lapso de tempo.

A "Diazona" mostrou-se mais hemolitica do que o "AMGL" e esta mais do que "Promin". Por outro lado, parece que a hemolise é diretamente proporcional á concentração de DDS livre no sangue e não á concentração total da droga, dando com isso uma avaliação de DDS livre no sangue, para o que ainda não existe método de dosagem.

A injeção de grandes doses (125 mg por kilo) resultou em grande concentração momentânea da droga no sangue no fim de 30 minutos. Depois de 4 horas havia pràticamente a mesma concentração sanguínea mesmo com

doses diferentes: 20 mg/k de "Diazona", 130 ou 65 mg/k de AMGL e 125 mg/k de "Promin".

Nenhuma das drogas nas doses utilizadas e dentro dos tempos observados, teve qualquer ação sôbre o número e volume das hemacias, nem sôbre os leucocitos. Não produziram também alteração sôbre a pressão carotidiana, com exceção do AMGL que teve muito leve ação depressora transitória.

Grandes doses de "Promin" injetadas na veia femural de cães não provocaram tromboses.

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